Mini-Review

Bacteria-mediated PAHs degradation in soil and sediment

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Abstract Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous in natural environment and easily accumulated in soil and sediment due to their low solubility and high hydrophobicity, rendering them less availability for biological degradation. However, microbial degradation is a promising mechanism which is responsible for the ecological recovery of PAH-contaminated soil and sediment for removing these recalcitrant compounds compared with chemical degradation of PAHs. The goal of this review is to provide an outline of the current knowledge of biodegradation of PAHs in related aspects. Over 102 publications related to PAHs biodegradation in soil and sediment are compiled, discussed and analyzed. This review aims to discuss PAHs degradation under various redox potential conditions, the factors affecting the biodegradation rates, degrading-bacteria, the relevant genes in molecular monitoring methods and some recent-year bioremediation field studies. The comprehensive understanding of the bioremediation kinetics and molecular means will be helpful for optimizing, monitoring the process and overcoming its limitations in the practical projects.

Key words Bioremediation, · Biodegradation,· PAHs, · Microorganisms

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a class of organic compounds with two or more fused benzene rings in linear, angular or cluster structural arrangements (Bamforth and Singleton 2005). They are produced from fossil fuel combustion, waste incineration, coal gastification and petroleum refining. PAHs are ubiquitous in the nature environments, including air (Bamforth and Singleton 2005), water (Zhou and Hua 2004), soil (Mulligan et al. 2001), sediment (Yuan et al. 2001), etc. These series aromatic compounds are of major concern since they are listed as priority pollutants by United States Environmental Protection Agency (USEPA) due to their toxicity to various organisms and their mutagenic and carcinogenic potentials to human through food chain (Mrozik et al. 2003).

Due to their low water solubility, high hydrophobicity and complex chemical structure, PAHs tend to accumulate in the soil and sediment, and have limited availability to biodegradation. However, biodegradation is still a major environmental process which affects the fate of PAHs in both terrestrial and aquatic ecosystems. Though the microbial degradation of PAHs of two or three rings has been well studied, the biological technology for remediation of PAHs-contaminated sites is not a very mature approach and still need more extensive studies (Bamforth and Singleton 2005). The successful application of bioremediation demands a broader understanding of the PAHs biodegradation process, including microorganisms, affecting factors, pathways, etc.

Since 2005, three reviews have been published on the biodegradation of PAHs (Bamforth and Singleton 2005; Peng et al. 2008; Haritash and Kaushik 2009). The latest review published in 2009 (Haritash and Kaushik 2009) focused on the biodegradation of PAHs by different microorganisms including bacteria, fungi and algae and summarized PAHs removals by composting, wetland and phytoremediation, most of which are based on the reports before 2004. The mini-review of 2008 summarized the PAHs degradation pathway (Peng et al. 2008). However, the important factors, such as surfactants and co-substrates, were not covered by these reviews. In addition, molecular techniques, which have been more extensively used in the study of bioremediation has not been reviewed. This review aims to provide a more comprehensive discussion on PAHs degradation under various redox potential conditions, the factors affecting the biodegradation rates, degrading-bacteria and the relevant genes in three parts. The scattered information in the 102 papers are discussed and summarized into five tables, i.e. PAHs biodegradation under nitrate-reducing (Table 1) and sulfate-reducing conditions (Table 2), surfactant effect on biodegradation (Table 3), aerobic degradation by various pure cultures (Table 4), and functional genes and the primers recently published for detection of PAHs-degrading bacteria (Table 5).

PAHs degradation under various conditions

Biodegradation can occur aerobically, anoxically or anaerobically. Numerous aerobic bacteria that utilize PAHs as carbon and energy source in the presence of oxygen have been isolated. Degradation of PAHs by aerobic bacteria under controlled conditions has been successfully applied in *ex situ* treatments of PAHs (Eriksson et al. 2003; Quantin et

al. 2005). However, researchers try to develop a series of *in situ* remediation technologies which offered numerous advantages including the operation cost and protection of ecological habitats and intrinsic microorganisms. Considering most of contaminated sediment and soil are under anoxic or anaerobic conditions, bioremediation of PAHs *in situ* is always associated with the anoxic/anaerobic biodegradation, of which the efficiency was affected by many factors, such as the nutrient limitation and other available carbon sources (Bach et al. 2005) (detailed in the session of "field studies of bioremediation of PAH-contaminated site"). In this part, PAHs biodegradation under various anoxic conditions is thoroughly discussed and compared. Table 1 and 2 summarized PAHs biodegradation under denitrifying and sulfate-reducing conditions, respectively.

Aerobic degradation of PAHs

An aeration system is always considered as a critical design component in large scale aerobic bioremediation, since oxygen supply is a rate-limiting factor. Aerobic bioremediation seems not cost-effective to implement due to the limitation of oxygen delivered to the subsurface for a couple of reasons, especially for sediment. Firstly, aeration will cause the re-suspension of sediment and the release of excess nutrient from sediment. Secondly, pure oxygen is expensive and low solubility, therefore, not cost-effective. Most of previous studies have focused on the aerobic degradation of PAHs due to the higher degradation rate and more-easily culturable aerobic pure bacteria, compared with anoxic/anaerobic biodegradation (Bregnard et al. 1996; Rockne and Strand 1998; Eriksson et al. 2003). However, hydrocarbon contaminated aquifers usually become

anoxic with a redox gradient along the groundwater flow path (Meckenstock et al. 2004). Therefore, in this review, aerobic biodegradation of PAHs is not thoroughly discussed, since aerobic bioremediation of sediment is not practical.

Denitrification

Nitrate-based bioremediation has been shown to be an attractive alternative to oxygen-based aerobic bioremediation which has been proved as a cost-effective approach for organic removal in sediment (Shao et al. 2009), since nitrate is more than ten thousand times higher soluble than oxygen in terms of electron accepting capacity and it can yield free energy almost as much as that under aerobic condition (MacRae and Hall 1998; Rockne et al. 2000). Moreover, nitrate (for example, calcium nitrate) is inexpensive and easy to be obtained. Therefore, *in situ* nitrate-based bioremediation has been more and more applied in the bioremediation of groundwater, soil and sediment contamination (MacRae and Hall 1998; Uribe-Jongbloed and Bishop 2007). Quite a number of studies were conducted to apply nitrate in remediation of sediment (McNally et al. 1998), soil (Eriksson et al. 2003; Dou et al. 2009) and sludge (Chang et al. 2003) by enriched cultures or pure cultures. Generally, in bench-scale studies, electron acceptors were directly mixed with the target biodegradation matrix in the bioreactors (Lei et al. 2005; Lu et al. 2010). In field studies, the electron acceptors as well as inoculums and nutrient were introduced by using an injection and abstraction well (Bamforth and Singleton 2005). Results compiled in Table 1 show that the low molecular weight PAHs, such as naphthalene and phenanthrene, were widely studied.

The results of Lei (2005) showed that under denitrifying conditions, no degradation of 16 PAHs in marine sediment was observed. Yuan and Chang (2007) reported that PAH degradation was inhibited under nitrate-reducing conditions, compared with that under sulfate-reducing condition. Such results could be attributed to the lack of PAH denitrifiers (Johnson and Ghosh 1998; Lei et al. 2005; Yuan and Chang 2007). Some researchers demonstrated the feasibility of using nitrate as an alternative electron acceptor to stimulate the growth of the heterotrophic denitrifiers and thus PAHs biodegradation in anoxic marine sediment (Zhang et al. 2009b). The half lives were 33-88 days for low molecular weight (LMW) PAHs and 143-812 days for high molecular weight (HMW) PAHs (MacRae and Hall 1998).

The study of Ambrosoli (2005) revealed that the presence of nitrate decreased PAHs degradation, probably because that PAHs biodegradation rate was higher under mixed denitrification and sulfate reducing conditions than that under only denitrification conditions (Ambrosoli et al. 2005). All other researchers suggested that PAHs biodegradation under denitrification conditions in soil-water systems may occur, although this may require acclimation periods of several weeks prior to the start of degradation (Mihelcic and Luthy 1988; Lei et al. 2005). The results of PAHs degradation in sludge under nitrate-reducing condition showed that the degradation rates were 0.005 mg/kg/d for pyrene and 0.035 mg/kg/d for phenanthrene, much lower than those under sulfate-reducing and methanogenic conditions (Chang et al. 2003). However, the reason for the low degradation rate under denitrifying condition is still not clear.

PAHs biodegradation by enriched cultures and pure cultures has also been intensively investigated. Rockne (2001) first demonstrated the nitrate-dependent degradation by pure culture. The degradation rate of naphthalene under nitrate-reducing conditions varied drastically, from 7.3 to 1434 μg/l/d, depending on the incubation temperature, electron acceptor (nitrate) concentration and operation conditions (with/without shaking) (Rockne et al. 2000). The highest degradation rate of 1434 μg/l/d was achieved by a mixed bacterial culture with the initial cell densities of 1×10^7 cells/ml (Dou et al. 2009). The degradation rate of phenanthrene ranged from 2.8 to 101.1 μg/l/d (MacRae and Hall 1998). The degradation kinetics was well described by first-order kinetic model or zero-order kinetic model (Bregnard et al. 1996; MacRae and Hall 1998).

Sulfate-reducing condition

In coastal marine sediment, oxygen and nitrate are less available in the subfacial layer, whereas sulfate is abundant. Therefore, PAH degradation coupled to sulfate reduction is the most associated to marine sediment. The feasibility of anaerobic degradation of PAHs under sulfate-reducing condition has been demonstrated in a number of investigations (Coates et al. 1996; Zhang and Young 1997; Lei et al. 2005). Table 2 summarized the studies of PAHs biodegradation conducted in sediment environment under sulfate-reducing conditions. Although the redox potential of nitrate is much higher than sulfate, there are still some studies showing that the degradation rates of PAHs were 0.14-0.583 mg/kg/d under sulfate-reducing condition, ten times higher than those (0.005-0.083 mg/kg/d) under nitrate-reducing (anoxic) condition (Chang et al. 2003). Moreover, it was found that PAHs biodegradation under sulfate-reducing condition had a long lag phase

which might be attributed to the high concentration of PAHs contamination in sediment (Zhang and Young 1997; Lei et al. 2005).

Other redox conditions

In some instances, Fe(Ⅲ) and Mn(Ⅱ) reduction are the dominant process in marine sediment environments. Bacteria using ion as electron acceptor have to cope with their extreme insolubility at a neutral pH. For example, soluble ferric-ion of 10^{-19} M is present in a saturated $Fe(OH)_3$ solution at the neutral pH (Seeliger et al. 1998). Therefore, ferric ion is quite insoluble and hence poorly bioavailable. It is noted that pH has an effect on mineralization of PAHs under Fe(Ⅲ) conditions and the mineralization rate of naphthalene under Fe(Ⅲ) conditions (174-292 μg/l/d) was lower than those under nitrate-reducing (301-552 μg/l/d) and sulfate-reducing (221-320 μg/l/d) conditions (Ramsay et al. 2003).

Effect of factors affecting the biodegradation of PAHs

Effect of surfactant

Surfactant-enhanced remediation has been suggested as a promising technology for the remediation of contaminated soil (Mulligan et al. 2001). *In situ*, surfactant is pumped into a contaminated site by introduction at an injection point and removal from an extraction point (Carriere and Mesania 1995). In laboratory studies, surfactant solution is directly mixed with PAHs in the reactors through stirring (Kim and Weber 2003). Table 3 lists the five groups of commonly used surfactants and their performance on PAHs

biodegradation, including nonionic, anionic, cationic, bio-surfactant and mixed-type surfactants.

Non-ionic surfactants

Non-ionic surfactants (NISs) are highly preferred and the most frequently applied in PAHs biodegradation as they are less toxic to bacteria than anionic and cationic surfactants (Boonchan et al. 1998; Jin et al. 2007). Among NISs, Triton X-100 was the most commonly investigated as shown in Table 3.

Effects of Triton X-100 on PAHs solubilization and biodegradation varied greatly. Triton X-100 at the concentration of 147×CMC (equivalent to 2.0 g/l) increased the naphthalene solubility from 6.4 mg/l to 80 mg/l (Kim et al. 2001). However, inhibitory effects of Triton X-100 were also observed, and the specific growth rates of naphthalene-utilizing and phenanthrene-utilizing bacteria strains decreased from 0.50 h^{-1} to 0.36 h^{-1} and from 0.30 h^{-1} to < 0.02 h^{-1}, respectively (Allen et al. 1999). Another research also reported that the residual phenanthrene was 63.5% of the initial value with the addition of Triton X-100 and 14.7% of the initial value in the absence of Triton X-100 (Avramova et al. 2008). In summary, the results of these studies showed that Triton X-100 may inhibit the degradation of PAHs to some extent although it may increase the solubility and bioavailability.

Quite a number of researches were conducted in a wide concentration range (0-1200×CMC) to evaluate the effects of Tween 80 on PAHs biodegradation. Tween 80,

another commonly used NIS, enhanced mobilization of the adsorbed PAHs at a high concentration of >20 g/l (Kim and Weber 2005). Fluoranthene degradation was enhanced from 62.4% without surfactant to 79.6% with 0.5 g/l (37×CMC) Tween 80 (Hickey et al. 2007). However, some inhibitory results of Tween 80 were also observed, k_1 (the first-order reaction rate constant) of phenanthrene degradation dropping from 0.504 d^{-1} without Tween 80 to 0.006 d^{-1} at 3×CMC Tween 80 (Wong et al. 2004).

Anionic and cationic surfactants

Most of earlier studies were performed with an anionic or cationic surfactant. However, with the frequent usage of such type surfactants, it has been found that they have many drawbacks in bioremediation of PAHs-contaminated soil. Cationic TDTMA surfactant inhibited the degradation of phenanthrene compared with anionic LAS surfactant (Jin et al. 2007). Anionic surfactants may precipitate in soil or in hard subsurface water (Zhao et al. 2005).

Some researchers (Penfold et al. 2002) found that the sorption amount of nonionic surfactant decreased with the increasing mole fraction of anionic surfactant in mixed solution. Therefore, cationic and anionic surfactants are suggested to be used with non-ionic surfactants for better performance.

Combined surfactants

Most of earlier studies were investigated with a single anionic or nonionic surfactant. However, in field applications, combined surfactants, rather than individual surfactants,

are often adopted, since the environmental factors (e.g. temperature, hardness, salinity and pH) have a certain effect on the solubilization of individual anionic or non-ionic surfactants for target compounds. For instance, anionic surfactants may precipitate in soil, while nonionic surfactants were more likely to adsorb onto clay fractions and thus would reduce the remediation efficiency and result in an increase in remediation time and costs. The studies on the effect of mixed anionic-nonionic surfactants on phenanthrene solubilization, desorption and biodegradation showed that the CMC values of all the three mixed surfactants, i.e. SDS-Tween80, SDS-Brij35, and SDS-TX100, were sharply lower than that of SDS and exhibited no inhibitory effect on biodegradation of phenanthrene (Zhao et al. 2005). SDS-Brij35 exhibited more significant degree of solubility enhancement for phenanthrene compared to SDS-TW80 and SDS-Triton X-100 (Zhao et al. 2005). However, SDS-TX100 enhanced or inhibited phenanthrene biodegradation at small or large ratio of SDS in the mixed solutions respectively, possibly due to the preferential utilization of SDS by phenanthrene degraders. The degradation rate in 24h for 1:9 SDS-TX100 mixed surfactants at concentrations of 1.0, 1.3, 1.6 and 1.9 mmol/l were about 115, 223, 165 and 132% of that for the single TX100, respectively (Zhao et al. 2005).

Biosurfactants

So far, only three studies with quite contradictive results have been reported about the biosurfactant effect on the PAHs biodegradation (Wong et al. 2004; Hickey et al. 2007; Avramova et al. 2008). Biosurfactant JBR could effectively increase the apparent solubility of PAHs and desorption from soil (Hickey et al. 2007). However,

biosurfactants P-CG3 and P.9027 produced by *Pseudomonas aeruginosa* inhibited phenanthrene biodegradation (Wong et al. 2004). The first-order reaction rate constant (k_1) of phenanthrene degradation decreased from 0.504 d^{-1} without P-CG3 to 0.007 d^{-1} at 3×CMC of P-CG3, and k_1 decreased from 0.504 d^{-1} without P.9027 to 0.005 d^{-1} at 3×CMC of P. 9027 (Wong et al. 2004). Another biosurfactant, JBR, increased the initial rate of fluoranthene degradation (Hickey et al. 2007). In general, biosurfactant-enhanced bioremediation technology is still at its early phase. There is growing interest in the use of biosurfactant for environmental applications, as synthetic surfactants are generally considered to be more toxic and require higher concentrations than biosurfactants.

Effect of nutrient

Microbial activity usually functions optimally at a certain carbon: nitrogen: phosphorus ratio (Zhou and Hua 2004). In contaminated sites, where organic carbon levels are often high due to the nature of the pollutant, available nutrients (N/P) can be rapidly depleted due to microbial utilization (Breedveld and Sparrevik 2000). Therefore it is common practice to supplement contaminated sediment with nutrients, generally nitrogen and phosphates, to stimulate the *in situ* microbial community and therefore enhance bioremediation (Breedveld and Sparrevik 2000). The levels of nutrient addition required for PAHs degradation are generally thought to be similar to those required for other organic pollutants such as petroleum compounds. So far, limited research has been done on the nutrient effect on the PAHs degradation in sediment or soil. However, it is found that amendment of inorganic N and P did enhance the level of PAHs degradation (Lei et al. 2005). It is demonstrated that concentrations of phenanthrene did not change

significantly without inorganic fertilizer (N, P), but decreased > 25 times in soil amended with N and P (Betancur-Galvis et al. 2006). Another study on co-substrates effect on PAHs degradation also proved that those additives with sufficient amounts of nitrogen, such as yeast extract and peptone, were more effective in enhancing phenanthrene degradation in contaminated sediment with C:N ratio of 100:1 (Chen et al. 2008).

Effect of co-substrate

The use of various co-substrates as readily degradable carbon sources aroused great interest in bioremediation of PAHs contaminated soil and sediment (Ortiz et al. 2003; Teng et al. 2010). Quite a number of compounds have been examined as co-substrates to study their effects on PAHs biodegradation, for example, acetate (0.5-0.86 g/l), lactate (0.5-1.46 g/l), pyruvate (0.5-1.74 g/l), humic acid (5 g/l, 3.36-10.08 g/kg soil), cellulose (0.00096-10 g/l), glucose, sucrose, peptone, methanol, ethanol and yeast extract (Yuan et al. 2001; Quantin et al. 2005; Liang et al. 2007; Chang et al. 2008). Six co-substrates, i.e. yeast extract, peptone, glucose, sucrose, ethanol and methanol, all at a concentration of 1 g/l, were compared on their effects on phenanthrene degradation (Kim et al. 2003). It was concluded that the order of effectiveness in enhancing phenanthrene degradation was: yeast extract> peptone >sucrose >glucose >methanol >ethanol (Kim et al. 2003). Besides the liquid form co-substrate, gaseous form could be an efficient way to enhance the biodegradation of PAHs in soil. The results showed that toluene with higher solubility than phenanthrene could induce the activation of enzymes that participate in the degradation of the target substrate and its intermediates (Ortiz et al. 2003). Generally, the easily-degradable co-substrates could increase the total population of bacteria at

remediated sites and thus enhance the bioremediation. Moreover, the co-substrate supplement could also change the C:N ratio, favor the microbial growth and increase the activity of the enzymes involved in the degradation of PAHs and their intermediates.

However, the effect of co-substrate could be adverse. It is reported that cellulose appeared to be a competing substrate and therefore inhibit the degradation of PAHs (Quantin et al. 2005). Sometimes, the addition of acetic acid (200 mg/l) and ethanol (150 mg/l) did not enhance the level of degradations of PAHs appreciably. Phenanthrene could be biodegraded with the supplement of pyruvate and be inhibited by succinate completely (Ortiz et al. 2003). Moreover, LMW PAHs were usually used as co-substrates to degrade HMW (four or five ring) PAHs. For instance, pyrene could not be degraded by *Burkholderia cepacia* 2A-12. But this strain could degrade pyrene when a co-substrate of naphthalene was supplemented (Kim et al. 2003). Fluorine degradation was inhibited by both phenanthrene and fluoranthene. The first order degradation rate for phenanthrene constants (k_1) at a concentration of 5 mg/kg decreased from 0.32 to 0.24 after adding acenaphthene of the same concentration but increased from 0.03-0.05 to 0.16-0.23 by other PAHs like fluorine, anthracene pyrene (Yuan et al. 2001).

When using readily degradable carbon source as a co-substrate in bioremediation, several factors need to be considered: (a) the optimal range of concentrations of the co-substrates; (b) the efficient way to distribute the co-substrate into the soil or sediment environment; (c) whether they can be completely mineralized without additional intermediates.

Effect of pH

An important factor for the biodegradation activity is the pH of the soil or sediment as it may affect the solubility and bioavailability of the pollutants and nutrients. Since the vast majority of bacteria exhibit optimal growth at or near neutral pH values, most laboratory-based biodegradation studies have been carried out in pH range of 5.0-9.0 (Yuan et al. 2001; Kim et al. 2003; Chang et al. 2008; Lu et al. 2010). It was reported that PAHs degradation was effectively enhanced after pH of the soil was adjusted from 5.2 to 7.0 by adding CaO although the degradation was not totally inhibited at pH of 5.2 (Kastner et al. 1998). Even smaller pH change also dramatically affected on the degradation of PAHs, one-unit pH shift changing the degradation rates of phenanthrene by four folds (Kim et al. 2005).

Effect of others

Except for the above-mentioned factors, quite a few researches were conducted on other influencing factors, such as humid acid (Liang et al. 2007), salinity (Kastner et al. 1998), particle size of sediment (Xia and Wang 2008), sediment/water ratio or soil/water ratio (Zhang and Bouwer 1997; Xia et al. 2006), organic content (Zhang and Bouwer 1997), physical mixing (Arzayus et al. 2002) and inoculation protocols (Kastner et al. 1998).. Elliott soil humic acid (ESHA) amendments at 20~200 μg ESHA/g soil were found to consistently increase pyrene mineralization by indigenous microorganisms while the dose of 10,080 μg ESHA/g inhibited and all other doses (from 400 to 3360 μg ESHA/g) had no effects (Liang et al. 2007). As to the particle size of sediment, the order of the

biodegradation rates of PAHs in water-sediment systems was fine silt > clay > coarse silt (Xia and Wang 2008). The biodegradation rate of PAHs increased with the sediment content in the water (Xia et al. 2006; Xia and Wang 2008). However, for soil, another research showed that high soil/water ratio decreased the degradation of PAHs (Zhang and Bouwer 1997). High salinity was suggested to inhibit PAHs degradation in the soil (Kastner et al. 1998). Intense physical mixing was found to contribute to more efficient removal of selected PAHs (Arzayus et al. 2002), as expected.

Microorganisms relevant to biodegradation of PAHs

Pure cultures of biodegradation of PAHs

Numerous studies have demonstrated that microorganisms play an important part in the bioremediation of PAHs-contaminated environments under various conditions. In the past two decades, a number of various bacteria have been identified as 'PAH-degraders', including the common genera of *Pseudomonas* (Weissenfels et al. 1990), *Sphingomonas* (Desai et al. 2008), *Cycloclasticus* (Geiselbrecht et al. 1998), *Burkholderia* (Kim et al. 2003), *Rhodococcus* (Di Gennaro et al. 2001), *Polaromonas* (Pumphrey and Madsen 2007), some novel genera of *Neptunomonas* (Hedlund et al. 1999) and *Janibacter* (Zhang et al. 2009a), some thermophilic bacteria of *Nocardia* (Zeinali et al. 2008) and *Bacillus* (Annweiler et al. 2000), some anoxic bacteria of *Deltaproteobacteria* (Musat et al. 2009) and *Alcaligenes* (Weissenfels et al. 1990), HMW PAHs-degrading bacteria of *Mycobacterium* (Šepič et al. 1998), *Stenotrophomonas* (Boonchan et al. 1998), and *Pasteurell* (Šepič et al. 1998). Table 4 lists the known pure cultures capable of degrading PAHs. It showed that most of isolates may degrade PAHs completely. Only one isolate of

Polaromonas naphthalanivorans CJ2 was inhibited by naphthalene. *Mycobacterium* sp. has been reported to be a common genus to degrade four or more ring PAHs. However it is not readily to be isolated and requires serial enrichment with pyrene as sole carbon and energy source.

In addition to bacteria, some fungi species can also degrade PAHs (Valentin et al. 2006). A combination of bacterial-fungal (*Mucor* sp. SF06 and *Bacillus* sp. SB202) degradation of PAHs has been suggested, and the removal percentage of Benzo[a]pyrene could reach 95.3% in 42 days while the Benzo[a]pyrene by fungi only did not result in any mineralization (Su et al. 2006).

PAHs metabolic genes

The genes encoding PAH-catabolic enzymes have been detected in a wide range of Gram negative bacterial and some Gram positive strains. The first step of PAHs degradation occurs *via* the incorportation of molecular oxygen into the aromatic ring by the ring-hydroxylating-dioxygenase (RHD) enzymes to form *cis*-dihydrodiols (Bamforth and Singleton 2005). The genes can be divided into two categories including *nah*-like genes of *Pseudomonas* sp. and non-*nah* genes which come from different bacteria (Habe and Omori 2003).

nah-like genes of Pseudomonas sp.

Almost all genes of this category come from *Pseudomonas* spp. Naphthalene degradation by *Pesudomonas putida* strain G7 has been well studied (LIoyd-Jones et al. 1999). These

catabolic genes are organized in three operons on the 83 kb plasmid, NAH7. The first operon encodes the upper-pathway enzymes involved in the conversion of naphthalene to salicylate, while the second encodes the lower-pathway enzymes involved in the conversion of salicylae to a TCA cycle intermediate *via* meta-ring cleavage and the third encodes a regulatory protein (NahR). Both upper and lower operons are regulated by a trans-acting positive control regulator encoded by the *nah*R gene. In this system, the genes encoding the terminal dioxygenase is highly conservative and composed of large α and small β subunits (Cebron et al. 2008). The α subunit contains two conserved regions: one is [Fe2-S2] Rieske centre and the other is the mononuclear iron-containing catalytic domain. Some researchers reported that because α subunit catalytic domain of *Sphingomonas* sp. is large enough to accommodate a five-ring benzo[a]pyrene molecule (Jakoncic et al. 2007), it can degrade this HMW PAHs. The most studied PAH dioxygenase (PAH-RHDα) is naphthalene 1,2 dioxygenase from *Pseudomonas putida* NCIB 9816-4, where the α subunit is encoded by the *nah*Ac gene. Based on the current studies, nahAc gene could be detected not only in the aerobic conditions but also some anoxic conditions (Lu et al., 2010). However, the successful amplification of a specific gene using these primers listed in Table 5 depends on many factors, including the universality of primers and existence of these genes. *nah*Ac gene was used to demonstrate the correlation between PAH biodegradation and the PAH-contamination level in the environmental samples by quantitative real-time PCR (Cebron et al. 2008; Lu et al. 2010), as a biomarker for PAHs degradation potential. PCR amplification using gene-specific primers has been used to investigate the diversity of PAHs dioxygenase

genes in PAH-degrading bacterial isolates or environmental samples. Table 5 summarized the reported primer sets used to detect PAH-degrading bacteria.

Other genes of various bacteria except for Pseudomonas strains

Other PAH-catabolic genes also exist in Gram-negative bacteria, including *phd* genes of *Comamonas teststeroni* strain GZ39 (Stach and Burns 2002), *nag* genes of *Pseudomonas* sp. strain U2 (Fuenmayor et al. 1998), and *phn* genes of *Burkholderia sp.* strain RP007 (Lloyd-Jones et al. 1999), and Gram-positive bacteria, including *nar* genes *Rhodococcus* sp. strain NCIMB12038 (Kimura et al. 2006) and *phd* genes of *Nocardioides* sp strain KP7 (Saito et al. 2000). All these genes above are related to degradation of naphthalene, phenanthrene and anthracene. The degradation of pyrene involves *nid* genes of *Mycobacterium* sp. strain PYR-1 (Zhou et al. 2006).

Comamonas testosteroni strains GZ39 was found to be able to degrade phenanthrene as the sole carbon source (Stach and Burns 2002). Cloning of *phd* genes in strain GZ39, which is responsible for the initial conversion of naphthalene and phenanthrene, demonstrated that this strain did not contain any genes similar to the classical *nah*-like genes from *P. putida* strain NCIB 9816-4. The *phd* gene in *Nocardioides* sp. strain KP7 was also characterized, and the results showed that all the *phd*ABCD genes were necessary for the efficient expression of phenanthrene-degrading activity (Saito et al. 2000).

nag genes encoding the naphthalene dioxygenase and involved in the conversion of salicylate into gentisate were sequenced and identified (Fuenmayor et al. 1998). Results suggested that the novel gene order *nag*Aa-*nag*G-*nag*H-*nag*Ab-*nag*Ac-*nag*Ad-*nag*B-*nag*F represented the archetype for naphthalene strains which use the gentisate pathway rather than the meta-cleavage pathway of catechol.

Burkholderia sp. strain RP007 was found to use naphthalene, phenanthrene and anthracene of LMW PAHs as the sole carbon source. The *phn* gene in this strain were reported to be different in sequence similarity and gene organization from the others genes (Lloyd-Jones et al. 1999). RNA analysis showed that the *phn* gene was detected in RNA rather than DNA, which implied that bacteria carrying the *phn* gene was present in low numbers in soil, but actively involved in degradation of naphthalene and phenanthrene (Stach and Burns 2002).

The genus *Rhodococcus* was reported to be able to utilize many aliphatic and aromatic compounds (Kimura et al. 2006). The *nar* genes in *Rhodococcus* sp. 1BN were sequenced and results revealed that a 59% nucleotide homology to the *Pseudomonas oleovorans alk*B gene. *nid* genes encoding a novel polycyclic aromatic-ring dioxygenase were cloned and sequenced from *Mycobacterium* sp. strain PYR-1, which is able to degrade pyrene, fluoranthene, phenanthrene, and benzo(a) pyrene (Moody et al. 2001). Recently, biodegradation potential of *Mycobacterium* populations in Lake Erie was assessed through real-time PCR quantification of pyrene dioxygenase genes of *nid*A encoding the

α subunit of dioxygenase and *nid*A was detected in all samples, ranging from 2.09 to 70.4 $\times 10^6$ copies per gram sediment (DeBruyn et al. 2009).

Field studies of bioremediation of PAH-contaminated site

Bioremediation of PAHs in the nature environment of field studies *in situ* is a complex process (Bewley and Webb 2001). The performance depends on a series of factors including the nature and concentration of the target compound, the seasonal environmental condition and the composition and activity of the indigenous microbial community (Smith et al. 2008). Moreover, the evaluation of bioremediation is far more difficult than in bench-scale studies. Several factors limiting the performance of bioremediation need to be considered including the low bioavailability and the PAHs distribution in the soil, difficulty in maintaining the optimum soil temperature and moisture for bacterial growth, elevated soil pH. In addition, evaluation of whether the target compounds are depleted needs to distinguish between the removal due to biodegradation versus abiotic effect such as sorption, volatilization and photodegradation (Maletic et al. 2009). In order to identify the specific process, one possible measure has been proposed that specific anaerobic metabolites produced during the degradation process could be used as the indicator of biodegradation. Table 6 summarized recent-year field work of bioremediation. The results indicated that in-situ treatment requires biostimulation and bioaugmentation as basic means. It is reported that the estimated cost of excavation, disposal and backfilling with clean soil is about $ 180,000 and the burial 1 m deep on site needs $100,000, while the bioremediation needs $38,000 (Andrea Leeson and Alleman 1999). Though the PAHs were biodegraded at a relatively slow rate in field

work and would have to operate several months to years to achieve the cleanup criteria, however, it is still a preferred option because of the cost and environmental friendliness.

Outlook

Degradation of PAHs by bacteria and fungi has been extensively studied over the past few years, leading to a deeper understanding of the biological degradation pathway and molecular genetics involved in the PAHs biodegradation bacteria. With the development of analytical chemistry, application such as LC/MS/MS and stable isotope analysis to this bioremediation study will lead to the identification of new pathways. Though many factors and many pure cultures discussed in this review have been optimized to elucidate the degradation mechanism, most of these studies were conducted under aerobic conditions. Further research is needed to optimize the parameters including co-substrate, surfactant, nutrient ratio, salinity etc. used in anoxic/anaerobic in-situ bioremediation that can be applied to remediate the subsurface area contaminated by PAHs, especially those HMW PAHs. In addition, further research is required to study the major microorganisms in PAHs degradation with the help of molecular techniques. The information on microbial community based on 16S rRNA and functional gene and functional gene are now available, however, the genes used for bioremediation are only present but not expressed. Therefore, the technology for quanlitifying mRNA concentrations is needed in future which is closely associated with contaminant degradation. Moreover, fluorescent *in situ* hybridization (FISH) with phylogenetic probes should be developed in the complicated *in situ* bioremediation to monitor the degradation process.

The main challenge for further study is to apply laboratory research results to the application in the practical field and to develop diagnostic technologies to determine which electron acceptors would be appropriate at a specific bioremediation site and enhance the mineralization of PAHs at a high degradation rate comparable with the aerobic conditions. Answers to these problems may help enhance understanding the mechanism of PAHs biodegradation, field scale PAHs bioremediation and might also formulate the risk assessment of PAHs-contaminated bioremediation engineering projects.

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Table 1 Biodegradation of PAHs under nitrate-reducing conditions

PAHs	Initial conc.[f] (μM)	Nitrate conc.[f] (mM)	Matrix	T[g] (°C)	Inoculum	Degradation rate (μM/d)	Source of PAHs	C^{14} label	Duration (d)	Reference
Naphthalene	54.7	1.2	Soil-water	25	Uncontaminated soil	0.95; 1.23; 1.17	Spiked	No	16-45	Mihelcic and Luty et al. 1988
	65.0	1.0	Soil	22±5	Aquifer	0.557	Spiked	Yes	100	Bregnard et al. 1996
	15.6	2.0	Artificial seawater	N.A.	Marine sediment	0.27	Spiked	No	57	Rockne et al. 2000
	1.2	1.4	Water	20-25	Contaminated sediment	0.02	Spiked	Yes	60	Rockne and Strand 2001
	3.9[a]	2.1-214.5	River sediment	25	River sediment	0.23	As is	No	50	Johnson and Ghosh 1998
	78.1	12.1	Northern soil	7	Soil	2.0	Spiked	No	40	Eriksson et al. 2003
	3781.3 [a]	44.2	Contaminated soil	36	Anaerobic digester sludge	9.6	Spiked	No	275	Uribe-Jongbloed et al.and Bishop 2007
	4139.1	80.6	Soil	25±0.5	Soil	N.A.	As is	No	315	Kim et al. 2008
	28.9-224.2	6.5	Water	20	Soil	1.45-11.2	Spiked	No	20	Dou et al. 2009
	6.3	3.5	Contaminated sediment	20	Contaminated sediment	2.4±0.5[d]	Spiked	No	30	Rockne and Strand 1998
	7.8-234.3	5.0	Seawater	25-45	Contaminated sediment	0.04-0.17[e]	Spiked	No	8	Lu et al. 2010
Acenaphthene	9.1[a]	2.1-214.5	River sediment	25	River sediment	0.14	As is	No	50	Johnson and Ghosh 1998
	48.7[a]	7.5	Marine sediment	25	Sediment	33-69[c]	Spiked	No	112	MacRae and Hall 1998
	6.5[a]	20.0	Water	30	Active sludge	0.06	Spiked	No	50	Chang et al. 2003
	6.5[a]	20.0	River sediment	30	River sediment	0.62	Spiked	No	72	Yuan and Chang 2007
Fluorene	60.2	12.1	Northern soil	7	Soil	0.78	Spiked	No	40	Eriksson et al. 2003
	6.0[a]	20.0	Water	30	Active sludge	0.22	Spiked	No	50	Chang et al. 2003
	6.0[a]	20.0	River sediment	30	River sediment	0.37	Spiked	No	72	Yuan and Chang 2007
Phenanthrene	1067.4	71.4	Marine sediment	20	Marine sediment	N.A.	As is	No	175	Lei et al. 2005
	1.0	1.4	Water	20-25	Contaminated sediment	0.022	Spiked	Yes	20	Rockne and Strand 2001

	56.2 [a]	2.1-214.5	River sediment	25	River sediment	0.79	As is	No	50	Johnson and Ghosh 1998
	5.1	1.0	Contaminated soil	20	Contaminated soil	0.42	Spiked	No	12-44[b]	McNally et al. 1998
	252.8[a]	7.5	Marine sediment	25	Sediment	43-55[c]	Spiked	No	112	MacRae and Hall 1998
	4.5	3.5	Contaminated sediment	20	Contaminated sediment	1.1±0.2[d]	Spiked	No	30	Rockne and Strand 1998
	5.6[a]	20.0	Water	30	Active sludge	0.22	Spiked	No	72	Chang et al. 2003
Anthracene	0.3	1.0	Contaminated soil	20	Contaminated soil	0.15	Spiked	No	12-80[b]	McNally et al. 1998
	101.1[a]	7.5	Marine sediment	25	Sediment	83-88[c]	Spiked	No	112	MacRae and Hall 1998
	5.6[a]	20.0	Water	30	Active sludge	0.10	Spiked	No	50	Chang et al. 2003
	5.6[a]	20.0	Water	30	Active sludge	0.24	Spiked	No	72	Chang et al. 2003
Fluoranthene	247.5[a]	7.5	Marine sediment	25	Sediment	143-157[c]	Spiked	No	112	MacRae and Hall 1998
Acenaphthene	48.7[a]	7.5	Marine sediment	25	Sediment	33-69[c]	Spiked	No	112	MacRae and Hall 1998
Pyrene	61.9[a]	2.1-214.5	River sediment	25	River sediment	0.79	As is	No	50	Johnson and Ghosh 1998
	0.6	1.0	Contaminated soil	20	Contaminated soil	0.3	Spiked	No	24-72[b]	McNally et al. 1998
	5.0[a]	20.0	Water	30	Active sludge	0.064	Spiked	No	50	Chang et al. 2003
	5.0[a]	20.0	Water	30	Active sludge	0.11	Spiked	No	72	Chang et al. 2003
Dibenzanthracene	251.8[a]	7.5	Marine sediment	25	Sediment	270[c]	Spiked	No	112	MacRae and Hall 1998

a: µmmol /kg ; b: hour: c: half lives (day) simulated by first-order.: d: specific PAH removal rate mg (g of VSS day)$^{-1}$;
e: mg-naphthalene/(lh); f: conc.: concentration; g: T: temperature; N.A. not available.

Table 2 Biodegradation of PAHs under sulfate-reducing conditions

PAHs	Initial Conc.[a] (μM)	Sulfate Conc[c]. (mM)	Matrix	T[d] (ºC)	Inoculum	Degradation rate (μM/d)	PAHs	C^{14} label	Duration (d)	Reference
Naphthalene	N.A.	10	Contaminated sediment	25	Contaminated sediment	N.A.	Spiked	Yes	60	Coates et al. 1996
	3.9[a]	0.5-52.1	River sediment	25	River sediment	0.12	As is	No	50	Johnson and Ghosh 1998
	50.8	28	Contaminated sediment	20	Contaminated sediment	0.43±0.06[b]	Spiked	No	30	Rockne and Strand 1998
Acenaphthene	N.A.	20	Water	30	Activated sludge	3.79	Spiked	No	50	Chang et al. 2003
	6.5[a]	20	Water	30	Activated sludge	2.55	Spiked	No	72	Chang et al. 2003
Fluorene	6.0[a]	20	Water	30	Activated sludge	2.95	Spiked	No	50	Chang et al. 2003
	6.0[a]	20	Water	30	Activated sludge	1.80	Spiked	No	72	Chang et al. 2003
Phenanthrene	56.2[a]	0.5-52.1	River sediment	25	River sediment	0.79	As is	No	50	Johnson and Ghosh 1998
	4.5	28	Contaminated sediment	20	Contaminated sediment	0.12±0.03[b]	As is	No	30	Rockne and Strand 1998
	5.6[a]	20	Water	30	Activated sludge	3.61	Spiked	No	50	Chang et al. 2003
	898.9[a]	30	Contaminated sediment	20	Contaminated sediment	3.17	As is	No	133	Li et al. 2005
	5.6[a]	20	Water	30	Activated sludge	1.02	Spiked	No	72	Chang et al. 2003
Anthracene	5.6[a]	20	Water	30	Activated sludge	1.80	Spiked	No	50	Chang et al. 2003
	5.6[a]	20	Water	30	Activated sludge	0.75	Spiked	No	72	Chang et al. 2003
Pyrene	61.9[a]	0.5-52.1	Water	25	River sediment	0.88	As is	No	50	Johnson and Ghosh 1998
	N.A.	20	Water	30	Activated sludge	1.16	As is	No	50	Chang et al. 2003
Anthracene	5.6[a]	20	River sediment	30	Activated sludge	0.43	As is	No	72	Chang et al. 2003

a: μmmol /kg ; b: specific PAH removal rate mg (g of VSS day)$^{-1}$; c: conc.: concentration; d: T: temperature; N.A. not available.

Table 3 Effects of surfactants on PAHs biodegradation

Surfactant	Conc.	Target compounds	Conc (mg/l)	Redox potential	Culture	Matrix	k^a	Performance	Reference
Tween80	675× CMC	Naphthalene/ Phenanthrene/Anthracene	500	Aerobic	*Pseudononas.sp. Enterobacter sp. Stenotrophomonas sp.*	Water phase	5.2×10^{-4}-2.0×10^{-2}	Greatly enhaced degradation	Bautista et al. 2009
	0-143× CMC	Phenanthrene	0-15	Aerobic	*Pseudononas putida* P2	Solution with phenanthrene	—	No toxic effect to bacteria	Jang et al. 2007
	19-112× CMC	Naphthalene, Phenanthrene	10-110,	Aerobic	Mixed	Soil slurry	5-31.2	Enhanced	Kim et al. 2001
	13-66× CMC	Phenanthrene	3.8-27.5	Aerobic	*Sphingomonas paucimobilis*	Water phase	0.066-0.091	Inhibitory effects	Kim and Weber 2003
	73×CMC	Phenanthrene	27.8	Aerobic	*Sphingomonas paucimobilis*	Water phase	0.559	No degradation	Kim and Weber 2005
	730-3650×CMC	Phenanthrene	110-115[c]	Aerobic	*Sphingomonas paucimobilis*	Soil phase	0.283-0.304	Inhibit the degradation	Kim et al. 2005
	0.5 g kg^{-1}	Fluoranthene	200[c]	Aerobic	*Pseudomonas alcaligenes* PA-10	Soil phase	1.46	Enhance the degradation	Hickey et al. 2007
	0-66×CMC	Phenanthrene	100	Aerobic	*Mycobacterium* spp. KR2	Solution with phenanthrene	0.018-0.115	Enhance the degradation	Jin et al. 2007
	1.5g/l	11 PAHs	5.5[c]	Aerobic	Sludge	Sludge phase	0.012-0.143	Enhance the degradation	Zheng et al. 2007
	0-3×CMC	Phenanthrene	230	Thermophiolic aerobic	*Bacillus* sp. B-UM	Water phase	0.006-0.504	Inhibit the degradation	Wong et al. 2004
Brij30	26-155× CMC	Naphthalene, Phenanthrene	10-110	Aerobic	Mixed	Soil slurry	10-44	No toxic effects	Kim et al. 2001
	0-93×CMC	Phenanthrene	N.A.	Aerobic	*Mycobacterium* spp. KR2	Solution with phenanthrene	N.A.	Enhance the growth of bacteria >40mg/L inhibition	Jin et al. 2007
Brij35	0-12×CMC	Phenanthrene	N.A.	Aerobic	*Mycobacterium* spp. KR2	Solution with phenanthrene	N.A.	Enhance the growth of bacteria >40mg/L inhibition	Jin et al. 2007
10LE	0-14×CMC	Phenanthrene	N.A.	Aerobic	*Mycobacterium* spp. KR2	Solution with phenanthrene	N.A.	Enhance the growth of bacteria >40mg/L inhibition	Jin et al. 2007
Triton X-100	73×CMC	Naphthalene/ Phenanthrene/Anthracene	500	Aerobic	*Pseudononas.sp. Enterobacter sp. Stenotrophomonas sp.*	Water phase	5.2×10^{-4}-2.0×10^{-2}	Greatly enhaced degradation	Bautista et al. 2009
	0-3×CMC	Phenanthrene	230	Thermophiolic aerobic	*Bacillus* sp. B-UM	Water phase	0.005-0.504	Inhibit the degradation	Wong et al. 2004
	0-37×CMC	Phenanthrene	0-15	Aerobic	*Pseudononas* putida P2	Solution with phenanthrene	N.A.	Toxic effect to bacteria	Jang et al. 2007
	18-110×CMC	Naphthalene, Phenanthrene	5-40	Aerobic	Mixed	Soil slurry	5.0-16.0	No toxic effects	Kim et al. 2001
	0.3-11.7×CMC	Naphthalene, Phenanthrene	5000	Aerobic	*Pseudomonas* sp., *Sphingomonas yanoikuyae*.	Micellar solution	Nap:<0.02[b]; Phe: 0.32-0.50[b]	Inhibitory effects	Allen et al. 1999
	5.8, 23.3×CMC	Naphthalene, Phenanthrene, Pyrene	21, 0.8	Aerobic	Mixed	Aueous phase and micellar phase	7.2, 0.216	Increase the degradation rate	Guha et al. 1998
	0-1000mg/l	Phenanthrene	5.5[c]	Aerobic	Mixed	Soil	33.3	Increase the bioavailability	Seo and Bishop 2007
	0-25×CMC	Phenanthrene	160	Aerobic	*Pseudomonas* sp.	Surfactant solutions	0.07	No effect on degradation	Avramova et al. 2008
Tergitol NP-10	100mg/l	Anthracene, Pyrene	1.0	Aerobic	Mixed	Micellar solution	0.022	Negative impact	Sartoros et al. 2005
	230×CMC	Naphthalene/ Phenanthrene/Anthracene	500	Aerobic	*Pseudononas.sp. Enterobacter sp. Stenotrophomonas sp.*	Water phase	5.2×10^{-4}-2.0×10^{-2}	Greatly enhaced degradation	Bautista et al. 2009

Tergitol 15S7	3-19×CMC	Phenanthrene	0.70-2.13	Aerobic	*Neptunomonas naphthovorans*	Saline water	0.108-0.47	Inhibit at high concentration	Li and Bai 2005
LAS	0-2.1×CMC	Phenanthrene	N.A.	Aerobic	*Mycobacterium* spp. KR2	Solution with phenanthrene	N.A.	Slightly increased below 10 mg/l	Jin et al. 2007
TDTMA	0-9×CMC	Phenanthrene	100	Aerobic	*Mycobacterium* spp. KR2	Solution with phenanthrene	3.5	Toxic to bacteria.	Jin et al. 2007
SDS-TW80	5.0-0.5; 2.0-0.5	Phenanthrene	27;18	Aerobic	Mixed	Surfactant solutions	7.6-9.0;	No toxic effect and enhance the degradation	Zhao et al. 2005
SDS-Brij35	5.0-1.0; 2.0-1.0	Phenanthrene	34;25	Aerobic	Mixed	Surfactant solutions	8.6-12.0	No toxic effect and enhance the degradation	Zhao et al. 2005
SDS-TX100	5.0-1.0; 3.0-1.0mM	Phenanthrene	20; 15	Aerobic	Mixed	Surfactant solutions	6.86-10.3	No toxic effect and enhance the degradation	Zhao et al. 2005
Rhamnolipid PS-17	1×CMC-0.004%	Phenanthrene	160	Aerobic	*Pseudomonas* sp.	Surfactant solutions	N.A.	No effect on degradation	Avramova et al. 2008
JBR Rhamnolipid	0.5 g kg^{-1}	Fluoranthene	200[c]	Aerobic	*Pseudomonas alcaligenes* PA-10	Soil phase	0.1209	Enhance the degradation	Hickey et al. 2007
P-CG3	0-3×CMC	Phenanthrene	230	Thermophiolic aerobic	*Bacillus* sp. B-UM	Water phase	0.007-0.504	Inhibit the degradation	Wong et al. 2004
P9027	0-3×CMC	Phenanthrene	230	Thermophiolic aerobic	*Bacillus* sp. B-UM	Water phase	0.005-0.504	Inhibit the degradation	Wong et al. 2004

a: *k* is the reported first-order kinetic rate constant;
b: specific growth rate h^{-1};
c: unit: mg/kg.

Table 4 PAHs biodegradation by aerobic pure cultures

Species	Incubation temp. (°C)	pH	Source	Substrate	Concentration (mg/l)	Performance	Reference
Alcaligenes denitrificans[a]	30	7.2	PAH-degrading mixed culture	Fluoranthene	1000	Degradation rate(mg/ml/d): 0.3	Weissenfels et al. 1990
Bacillus thermoleovorans Hamburg 2	60	6.5	Contaminated compost	Naphthalene	4.7 9.5	77.4% degradation 82.2% degradation	Annweiler et al. 2000
Burkholderia cepacia 2A-12	30	N.A.	Oil-contaminated soil	Naphthalene and phenanthrene	215	Degradation rate: 11.14μmol $l^{-1}h^{-1}$	Kim et al. 2003
Cycloclasticus W	20	N.A.	Marine sediment	Naphthalene	5	100% degradation in 7 days	Geiselbrecht et al. 1998
Deltaproteobacteria NaphS3[b]	N.A.	N.A.	Sediment	Naphthalene	20	unknown	Musat et al. 2009
Gordonia sp. strain BS29	25	N.A.	Diesel-contaminated soil	Phenanthrene Anthracene Pyrene	183 312 178	96.1% 39.4% 97.8% degradation in 76d.	Franzetti et al. 2009
Janibacter anophelis JY11	30	7	Oil-polluted soil	Phenanthrene Anthracene Pyrene	500	98.5%, 82.1%, 97.7% degradation	Zhang, et al. 2009
Mycobacterium sp. 1B	30	6.9-7.0	A bacterial culture capable of benzo[a]pyrene	Fluoranthene Phenanthrene Pyrene	100 250 250	100% degradation within 5-6 days 100% degradation within 5-6 days 100% degradation within 18-20 days	Dandie et al. 2004
Mycobacterium sp. PYR-1	22-26	N.A.	Oil-contaminated soil	Fluoranthene	20	46% degradation in 14 days	Šepič et al. 1998
Neptunomonas naphthovorans NAG-2N-126	20	N.A.	Creosote-contaminated sediment	Naphthalene, Phenanthrene	5	Both 100% degradation in 7 days	Hedlund et al. 1999
Nocardia otitidiscaviarum TSH1	50	6.8	Petroindustrial wastewater contaminated soil	Naphthalene	500	Unknown, benzoic acid as an intermediate	Zeinali et al. 2008
Pasteurella sp. IFA	22-26	N.A.	Oil-contaminated soil	Fluoranthene	20	24% degradation in 14 days	Šepič et al. 1998
Pseudomonas citronellolis 222A	30	7	Landfarm used for effluent treatment from petro chemical industries and oil refinery	Anthracene	250	72% degradation in 48 days with 0.1 mM iron stimulated	Santos et al. 2008
Pseudomonas paucimobilis	30	7.2	PAH-degrading mixed culture	Phenanthrene	1000	Degradation rate(mg/ml/d): 1.0	Weissenfels et al. 1990
Polaromonas naphthalanivorans CJ2	N.A.	7.4	Coal-tar contaminated freshwater sediment	Naphthalene	10	Inhibited by naphthalene	Pumphrey and Madsen 2007
Rhodococcus opacus R7	30	N.A.	PAH-contaminated soil	Naphthalene	1000	Unknown, salicylic acid as an intermediate	Di Gennaro et al. 2001
Sphingomonas paucimobilis EPA505	30	N.A.	Creosote facility	Fluorene Naphthalene	0.36 0.79	q_{max}=0.04[c] q_{max}=0.10[c]	Desai et al. 2008
Stenotrophomonas maltophilia VUN 10, 010	30	N.A.	Gas manufacturing plant soil	Pyrene	250	100% degradation in 49 days	Boonchan et al. 1998
Streptomyces griseus	29	N.A.	-	Naphthalene	600	100% degradation in 144 h	Gopishetty et al. 2007

a: denitrification species; b: sulfate-reducing bacteria species; c: degradation rate (mg substrate/mg protein/h).

Table 5 Primers of PAHs-degrading genes

Primer	Sequences (5'→3')	Location	Target gene	Annealing temp. (°C)	Size of product (bp)	Reference
NDO 355F NDO 924R	TTY GAA AAA GAG YTG TAC GG TTC GGG AAA ACS GTG CAG TT	355 924	*nah*AaAb *nah*AcAd	48	569	Ma et al. 2006
nah-for *nah*-rev1 *nah*-rev2 (nested)	TGC MVN TAY CAY GG YTG G CCC GGT ARW ANC CDC KRT A CRG GTG YCT TCC AGT TG	N.A.	*nah*Ac *phn*Ac *nag*Ac	55	937/317	Zhou et al. 2006
PAH-RHD$_\alpha$ GN F PAH- RHD$_\alpha$ GN R	GAG ATG CAT ACC TKG GTT GGA AGC TGT TGT TCG GGA AGA YWG TGC MGT T	610 916	*nah*Ac	57	306	Cébron et al. 2008
*nah*Ac-1F *nah*Ac-1R	AAG AGC TGT ACG GCG AGT C CCT GAT CGA AGC AAC CAT AG	362 444	*nah*Ac	55	102	Park and Crowley 2006
*nah*Ac-3F *nah*Ac-3R	GAC GCT GCT TGG TAC CTA GA TCC AGT TGG CCT TGA TCA	493 569	*nah*Ac	55	94	Park and Crowley 2006
*nah*Ac-6F *nah*Ac-6R	TGA TCA AGG CCA ACT GGA AGC GAC GCG AAG ATA GAC TC	569 661	*nah*Ac	55	112	Park and Crowley 2006
*nah*Ac-7F *nah*Ac-7R	ACT TGG TTC CGG AGT TGA TG CAG GTC AGC ATG CTG TTG TT	791 907	*nah*Ac	55	136	Park and Crowley 2006
*nah*Ac-F *nah*Ac-R	TGG CGA TGA AGA ACT TTT CC AAC GTA CGC TGA ACC GAG TC	63 1072	*nah*Ac	55	992	LIoyd-Jones et al. 1999
*nah*Ac F *nah*Ac R	TGA GTG AAT CTG GGC TG ATC CTC GAA CTC AGC C	N.A.	*nah*Ac	N.A.	N.A.	Fuenmayor et al. 1998
*nah*Aa F *nah*Aa R	TCA TAC AGC CAA ACA ATC GAT AGA AGG CAT CGG	N.A.	*nah*Aa	N.A.	N.A.	Fuenmayor et al. 1998
*nah*Ab F *nah*Ab R	ACT GTC GAG GGC AAG ATT ACG CGC AGG TTC TC	N.A.	*nah*Ab	N.A.	N.A.	Fuenmayor et al. 1998
*nah*Ad F *nah*Ad R	ATT CAA GAA GAC AAG CTG GTA ATC CAC GAA TCG CTG	N.A.	*nah*Ad	N.A.	N.A.	Fuenmayor et al. 1998
phn F *phn* R	TTC GAG CTG GAA TGT GAG C AAT AAC CGG CGA TTC CAA AC	66 1076	*phn*Ac	55	993	LIoyd-Jones et al. 1999
*nag*G *nag*Ab	GGA TAC CAA CAT ATG AGT GAA CCC CAA C CAT AAA TCA TGA TTA ATG TCT CCG TT	1290 3458	*nag*G,*nag*H, *nag*Ab	N.A.	N.A.	Fuenmayor et al. 1998
*nar*Aa F *nar*Aa R	AAGCTCGGCGCAGACAACTTC TAGTTGAGCTCCCCTGTCTTG	4510 5916	*nar*Aa	N.A.	574	Kimura et al. 2006
*nar*Ab F *nar*Ab R	GAC GTC GTC GAG GGA ATG AGC AGC AGG TTC GAG GTG	5920 6438	*nar*Ab	N.A.	187	Kimura et al. 2006
*nar*B F *nar*B R	ATC TCC CCG GAG AAG GTG CGT TGA CGC CGA AGA GTT	6452 7543	*nar*B	55	240	Kimura et al. 2006
*nid*A-F *nid*A-R	CCT TAT GTC CAG GGC TTC A TAG CGA CTC CGA CTT CAC G	1208 1344	*nid*A	57	136	Debruyn et al. 2009

Table 6 Field studies of bioremediation of PAH-contaminated sites

Bioremediated sites	Contaminant	Concentration	Operation	Remediate period	Degradation rate	Field results	Reference
PAH-contaminated groundwater, UK	PAHs and BTEX	11 μg/l	Supplies N&P , inoculum and nitrate	2.5 year	0.01 μg/l/d	93.6% removal	Bewley and Webb 2001
PAH contaminated soil, Australia	PAHs	174 mg/kg	Landfarming and intrinsic bioremediation	1.5 year	18.9 mg/kg/d	Over 33% biodegraded	Andrea Leeson and Alleman 1999
PAH contaminated soil, USA	PAHs	7300 mg/kg	landfarm bioaugmentation	16 months	13.0 mg/kg/d	86-87% degradation	Nestler et al. 2001
PAH contaminated sediment, USA	PAHs	<200 mg/kg	Supply N/K at 3/6 mg/kg	3.0 year	N.A.	LMW 94-100%; HMW 0-30%	Smith et al. 2008
PAH contaminated soil, Serbia	PAHs, oil and hydrocarbons	3.57 mg/kg	Biopile	500 days	$6.7\text{-}8.8\times10^{-3}$ [a]	77% removal	Maletic et al. 2009
Gas plant, NY, USA	PAHs	333.7 mg/kg	Natural attenuation	2.0 year	0.003-0.15 [a]	Toxicity decreases	Robert E. Hinchee et al. 1995
Creosote contaminated soil, USA	PAHs	1300 mg/kg	Land farming	4 months	0.01-0.015 [a]	84.6T removal	Andrea Leeson and Alleman 1999

a: first-order biodegradation kinetic constant rates